UniMab™ and UniMab™ HC
-Monodisperse Protein A Affinity Resins

Product Description

UniMab™ and UniMab™ HC are rigid protein A affinity resins designed for the downstream purification of monoclonal antibodies (mAb) and Fc-fusion proteins. Their support matrix is a highly crosslinked polymethacrylate resin of uniformed particle size and open pore structure. The matrix surface is derivatized with hydrophilic polyhydroxylated layer that is further coupled with recombinant Protein A affinity ligand through epoxy chemistry. Both UniMab™ and UniMab™ HC are figured with exceptional mechanical strength, excellent pH stability, low leachates, high recovery yield, and rapid mass transfer. UniMab™ HC exhibits better dynamic binding capacity than UniMab™ due to ligand and surface chemistry improvement. Table 1 lists the basic characteristics of these two resins, while Figure 1 displays the nature of monodisperse particle size distribution of the resins.

Table 1. Characteristics of UniMab™ resin

Characteristics	Specification	
	UniMab™	UniMab™ HC
Separation Function	Protein A affinity	
Matrix	Rigid, monodisperse Polymetharylate bead	
Ligand	Engineered Recombinant Protein A	
Coupling Chemistry	Epoxy	
Particle Size（μm）	50 μm	
Dynamic Binding Capacity (hIgG)	~ 40 mg/mL	~50 mg/mL
Protein A Leachate	< 5 ng/ml	
Pressure Resistance	145 psi（ 10 bar, 1.0MPa ）	116 psi (8 bar, 0.8 MPa)
pH working range	3 - 12	
CIP	0.1 - 0.5 M NaOH	
Temperature Stability	4 ~ 40 °C	
Storage	20% ethanol，2 – 8 °C	
Chemical stability	Stable in all aqueous buffers commonly used in affinity chromatography	

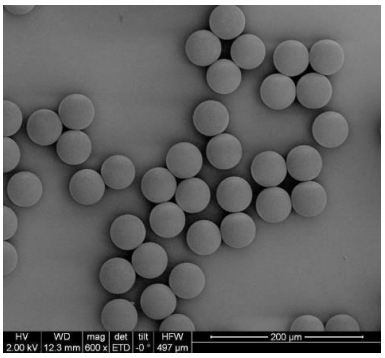


Figure 1. Representative microscopy image of UniMab™ resin. Note the mono-sized nature of the resin.

Product Highlights

- **Superior Pressure-Flow Characteristic**

UniMab™ and UniMab™ Pro are based on rigid matrix of high mechanical strength. It can tolerate >1.0MPa pressure that is much higher than conventional bioprocessing resins' pressure limits. It exhibits excellent linear pressure vs flow rate relation even at very high flow rate (Figure 2). This exceptional characteristic enables high flow rate bioprocessing purification for productivity improvement.

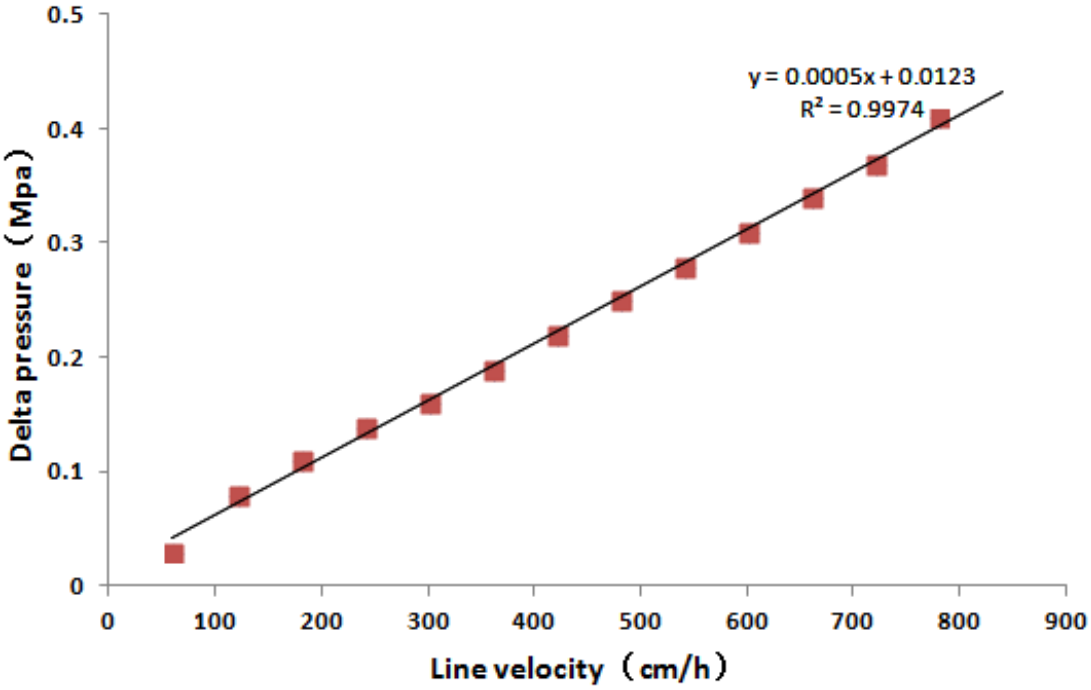


Figure 2. Pressure/flow curves of UniMab™ (Test column: 1.6×22cm; mobile phase: 20 mM PBS, 0.15 M NaCl; temperature:25°C).

- **High Dynamic Binding Capacity at High Flow Rate**

Due to its open pore structure for fast mass transfer, UniMab resin exhibits higher DBC than conventional agarose type Protein A resin at short residence time (Figure 3).

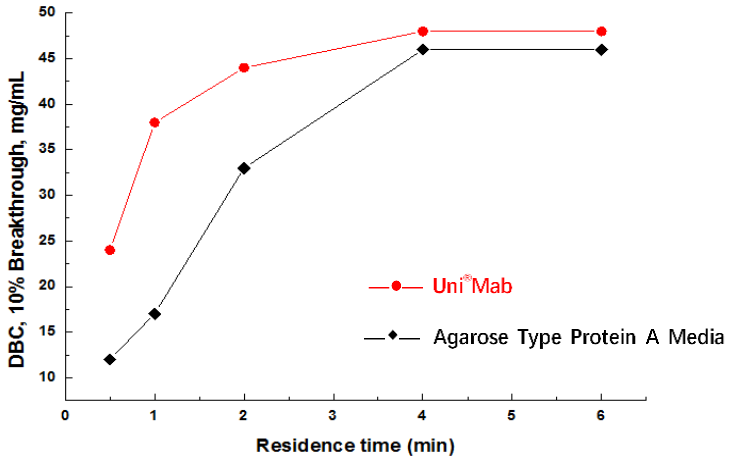
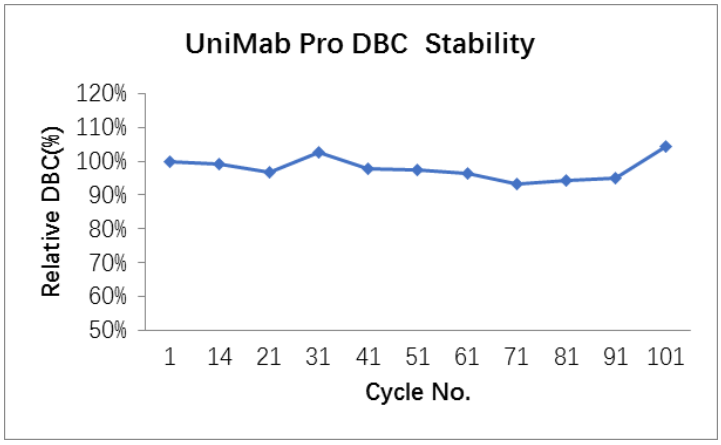


Figure 4. hIgG DBC of UniMab™ vs a agarose resin at varied residence time.

- **Excellent Stability and Low Protein A Leachate**

Both UniMab™ and UniMab™ HC have shown excellent stability. The following figures (Figure 5) display these two resins' DBC performance at various cycles of IgG capture. Each cycle contains a 5CV CIP step using 0.1M NaOH. After 100 cycles of use, its DBC did not significantly change.



Steps	Buffer	CVS	RT(min)
Equilibration	20mmPB+150mm NaCl, PH7.4	5	5
Load	hIgG 2.5mg/ml	10ml	5
Wash	20mmPB+150mm NaCl, PH7.4	5	5
Elution	50mmHAC-NaAC，PH3.5	5	5
Stip	1M HAC	5	6
Wash	20mmPB+150mm NaCl, PH7.4	3	5
CIP	0.1M NaOH	5	6
Equilibration	20mmPB+150mm NaCl, PH7.4	5	5

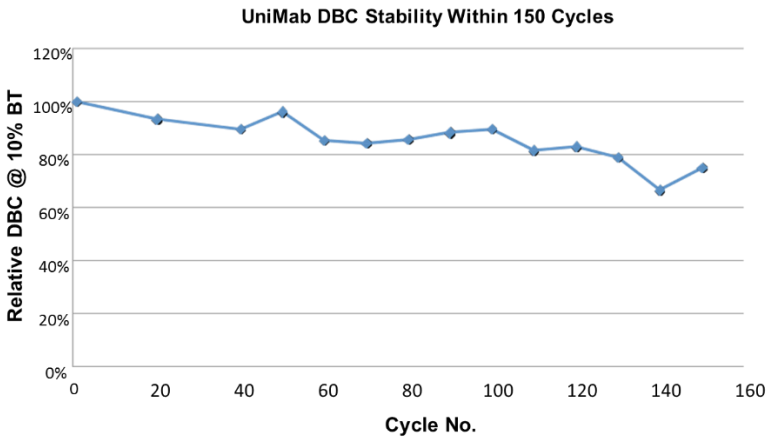


Figure 5. DBC of various cycles of use in IgG capturing.

Column Packing

UniMab™ and UniMab™ HC are supplied in 20% Ethanol preservative solution. Prepare the resin slurry by removing the storage solution and replace it with the packing solvent. Recommended packing solvent is a 0.1 M NaCl solution. The 1.07±0.02 compression factor is recommended to account for the difference in bed volume between a gravity-settled bed in 0.1 M sodium chloride and a 3 bar pressure packed bed. This factor, along with the slurry ratio, is used to determine the volume of slurry required to yield the intended final column volume.

1. Pour the slurry into the column, for best results the column should be filled in one time at a smooth steady rate. Typically this is accomplished by pouring down a glass rod held against the wall of the column that helps prevent the introduction of air bubbles.
2. Fill the remaining column space and reservoir with 0.1 M NaCl. Place the lid on the packing reservoir and connect it to the pump.
3. Open the column outlet and start the packing by pumping 0.1 M NaCl through the column at a slow flow rate (60 cm/h) until the bed height stabilizes.

4. Increase flow rate and flow at constant pressure (0.5-0.7 MPa) for about 30 min.
5. Switch of and disconnect the pump. Close the column outlet.
6. Remove the packing reservoir (this is best done over a sink or drain). Refill the column to the top with 0.1 M NaCl.
7. Wet the column adaptor by submerging the plunger end in 0.1 M NaCI, and drawing through with a syringe. Ensure that all bubbles have been removed.
8. Insert the adaptor into the top of the column, taking care not to introduce air bubbles.
9. With the adaptor outlet open, push the adaptor into the column and down onto the resin bed, allowing the 0.1 M NaCl to displace any air remaining in the tubing.
10. Lock the adaptor in place, connect it to the pump, open the column outlet and continue packing at a flow rate equivalent to the process flow rate (< 5 bar) for 3 minutes.
11. Mark the position of the top of the bed height on the column cylinder wall. Stop the pump, close the column outlet and reposition the adaptor to approximately 1 mm below the marked bed height position.

Resin Cleaning and Storage

Cleaning-in-place (CIP)

The following ClP protocols are intended as a starting point cleaning protocol specific for a given feed material. Typically, ClP is conducted every 5 cycles but this will ultimately depend on the nature of the feed material. Different contaminants require different or even combine ClP protocols. Severe fouling will require specific protocol development.

Precipitated or denatured substances:

- Wash with 2 - 4 column volumes of 0.5 M NaOH.
- Wash immediately with at least 5 column volumes of 0.2 μ filtered binding buffer at pH 7-8.
- Reverse flow direction.

Hydrophobically bound substances:

- Wash the column with 2 column volumes of a non ionic detergent1 (e.g. conc. 0.1%).
- Wash immediately with at least 5 column volumes of sterile filtered binding buffer at pH 7-8.
- Reverse flow direction.

OR

- Wash the columns with 3-4 columns volumes of 30% isopropanol1.
- Wash immediately with at least 5 columns volumes of sterile filtered binding buffer at pH 7-8.
- Reverse flow direction.
- Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.

Resin Storage

Unused resin can be stored in the container at a temperature of +2 to +8 ℃. Ensure that the screw top is fully tightened. Packed columns should be equilibrated in binding buffer containing 20% ethanol to prevent microbial growth. After storage, equilibrate with at least 5 bed volumes of starting buffer before use.

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